"On certain Physical and Chemical Properties of Solutions of Chloroform in Water, Saline, Serum, and Hæmoglobin. A Contribution to the Chemistry of Anæsthesia.—(Preliminary Communication.)" By Benjamin Moore, M.A., D.Sc., Johnston Professor of Bio-chemistry, University of Liverpool, and Herbert E. Roaf, M.B., Toronto, Johnston Colonial Fellow, University of Liverpool. Communicated by Professor C. S. Sherrington, F.R.S. Received April 12,—Read May 5, 1904.

The number of substances which have been shown to possess more or less well-marked anæsthetising properties reaches some hundreds, and hence it is obvious that the action cannot have a different explanation in each case, but rather depends upon some general type of interaction between the anæsthetic and the active part of the cell, which is the cell-protoplasm.

Further, the action occurs not only with nerve-cells, but with ciliated and other epithelial cells, with muscle-cells of all types, with bacteria, amœbæ, and other unicellular organisms, and with all types of vegetable cells in which activity is suited to experimental demonstration. In all these varied types of living cell, activity decreases alike with increasing dose of the anæsthetic, and, with sufficient concentration, all sign of life becomes obliterated.

Hence the action of the anæsthetic must be due to some change brought about in the only material which is uniformly present in all these types of cell, that is, the cell-protoplasm.

Accordingly, in briefly reviewing, as an introduction to our experiments, the previous theories of anæsthesia which have been advanced by various observers, we believe we may justly cast aside those which attribute it fundamentally to anything peculiar in the structure or chemical composition of the nerve-cell, or to any alteration in the nutrition of the nervous system, brought about by variations in its blood supply or otherwise.

It is true that cells differ in the degree of their reaction to anæsthetics, but not in kind, and ultimately the metabolic processes of bacteria are stilled as effectually as are those of the mammalian nerve-cell. Any such effects as anæmia or hyperæmia of the brain, which have been alternately described by various observers, must accordingly be only set down as secondary effects, and not as primary causes of anæsthesia.

Similarly, theories which are based on the peculiarly high content in cholestearin, lecithin, and fatty derivatives soluble in ether, of the nerve-cell and its processes, cannot furnish an explanation of anæsthesia,

for these substances are not present in demonstrable quantity in by far the greater number of animal and vegetable cells.

Turning to the views of anæsthesia which rest upon an interaction between the anæsthetic and the cell-protoplasm, we find the speculation first thrown out by Claude Bernard* in 1875, that anæsthesia consists in a semi-coagulation of the substance of the (nerve) cell, a coagulation which may not be definite, that is to say, in which the substance can return structurally to its primitive state after elimination of the toxic agent. Bernard supports his view chiefly by analogy, and instances the stiffening and opacity of skeletal muscle when exposed to chloroform vapour.

A similar view was expressed by Binz,† who stated that sections of cerebral cortex placed in 1-per-cent. solution of hydrochlorate of morphia soon showed a cloudy appearance, and fine granules appeared in the nuclei; the protoplasm also became granular. The stage at which the cell-protoplasm was merely cloudy, and not discretely granular, could be recovered from by washing away the morphia, but, when once the granules appeared, they could not be made to disappear again. Similar results were obtained by exposing cortical nerve-cells to vapour of chloroform, or to solution of chloral hydrate.

Neither Binz nor Bernard showed, however, that there was any precipitation or semi-coagulation at or near the concentrations which correspond to anæsthesia, nor were the optical methods used capable of demonstrating effects upon the protoplasm short of precipitation.

Similar speculations of a *general* nature regarding the action of toxic agents, as being due to the formation of a loose, easily-dissociated compound between the toxic agent and the cell-protoplasm, have been thrown out by various writers, as, for example, Buchheim (1856) and Schmiedeberg (1883).

Demoor‡ has shown that, subsequent to prolonged and deep anæsthesia, the dendrites of nerve-cells acquire moniliform swellings, and has founded on this a mechanical theory, which rests on the view that the swellings observed are due to a retraction of the protoplasm of the dendrites, so that the communication of cell with cell is interrupted.

The swellings described by Demoor have also been observed by Hamilton Wright,§ who also found that they became larger, more numerous, and encroach more and more on the dendritic stems the longer the anæsthesia is kept up.

These effects are of importance as evidence of an interaction between protoplasm and anæsthetic, but the retraction theory of Demoor based

* 'Leçons sur les Anesthésiques,' etc., 1875, p. 153.

† 'Vorlesungen über Pharmakologie,' p. 175.

‡ 'Arch. de Biol.,' 1896, vol. 14.

§ 'Journ. of Physiology,' vol. 26, 1900, p. 30; vol. 26, 1901, p. 362.

on them will not hold in view of more recent work on the fibrillar mode of communication between cell and cell. Further, as pointed out above, any adequate view as to how anæsthetics produce their effect must be applicable also to the unicellular organism, and not merely to the nerve-cell, or any colony of cells.

Wright obtained further effects as a result of prolonged anæsthesia, which led him to adopt the view that the action is bio-chemical in character. He found, for example, that the Nissl's bodies lost their affinity for basic dyes, such as methylene blue, but that this effect was only temporary, and disappeared as soon as the anæsthesia had passed off.

These changes, however, do not begin to appear immediately the anæsthetic commences to produce its effect, and are rather a signal of the changes produced in the protoplasm in marked degree by the prolonged action of the anæsthetic than an indication of the first reaction between cell-protoplasm and anæsthetic.

Our own attention was first attracted in this direction by witnessing the experiments of Sherrington and Sowton* upon the effects of chloroform on the excised mammalian heart, fed by a current of Ringer's or Locke's solution, and through which later a similar current, but containing in addition small amounts of chloroform, could be perfused.

These authors observed that a concentration of chloroform in the Locke solution, amounting to only 1 in 100,000, produced a marked and unfailing action in diminishing the extent of the cardiac contractions, and further that this effect appeared rapidly after the dilute chloroform solution reached the heart, lasted just as long as the chloroform in this excessively low but yet adequate concentration was passed through, and ceased almost immediately as soon as the normal Locke's solution was recurred to, the heart attaining again its normal force.

This effect could be repeated as often as was desired, and there was no cumulative action whatever, that is, no matter how prolonged the passage of the chloroform solution on passing back to the normal Locke's solution, the chloroform effect rapidly disappeared, and again recovered when the chloroform was once more turned on.

It was this latter effect which suggested the experiments on the chemistry of anæsthesia recorded in this paper. It was quite obvious that the effect of the chloroform upon the cardiac muscle fibres depended solely upon the concentration (solution tension, or osmotic pressure) of the chloroform in the cell for the time being, and not at all upon the total amount of chloroform which had been fed to the heart up to the moment of observation.

This experimental fact suggested the view that the effect upon the

* Thompson Yates, 'Johnston Laboratories Reports,' vol. 5, Part 1, p. 69.

cell was due to some combination being formed between the protoplasm and the chloroform, and further that this combination was not a stable fixed one, leading to permanent removal of the protoplasmic activity, but an unstable one, which existed only so long as the pressure of the anæsthetic was kept up to a definite level, and gradually dissociated as the level of chloroform pressure was allowed to fall, and as a result left the protoplasm free for a renewal of its metabolic processes—to choose a familiar analogy, that the protoplasm of a cell undergoing anæsthetisation entered into a combination with the anæsthetic, similar to that between hæmoglobin and oxygen, unstable in character, and only lasting so long as the pressure of the anæsthetic was kept up.

It occurred to us, as protoplasm is built up chemically of proteid, that a certain amount of evidence as to the formation of such an unstable compound might be obtained, in the first instance, by experimenting with proteids.

We accordingly experimented with the proteids of the blood, and have obtained a number of results which together point to the formation of such compounds as are indicated above.

It is our intention to proceed further and study in a similar fashion the effects of chloroform upon various types of living cell, but we here present the work done upon proteids, which appears to us to prove that an easily dissociable compound is formed between proteid and chloroform.

Our experiments may be described under the following headings :—

1. On the obvious physical and chemical changes produced in serum and in hæmoglobin solution by the addition of chloroform.

2. On the relative solubility of chloroform in water, normal saline solution, serum, and hæmoglobin solution.

3. On the relative vapour pressures of chloroform when dissolved in water, saline, serum, and hæmoglobin solutions respectively, and on the variations in the coefficient of distribution in these solutions.

4. On the solubilities of gases in serum and hæmoglobin solution in presence of chloroform.

I.—*Effects of Chloroform on Serum and on Hæmoglobin Solution.*

On adding chloroform* to either serum or hæmoglobin solution, and allowing the mixture to stand, changes occur which are obvious to the eye, and were to us previously unknown, but on consulting the literature we found that they had been observed by E. Salkowski† in

* The chloroform used for all the experiments described in this communication was presented to us by Messrs. Duncan and Flockhart, of Edinburgh.

† 'Deutsche Med. Wochensch.,' 1888, No. 16; 'Zeitsch. f. Physiol. Chem.,' vol. 31, 1900, p. 329. The fact that the red blood corpuscles combine with chloroform is also mentioned by Schmiedeberg, 'Arch. f. Heilkunde,' 1867, p. 273.

using chloroform as a preservative for these fluids, and were also described by Formánek,* the bearing of such phenomena upon the question of anæsthesia was not, however, appreciated by these previous observers, who had approached the matter from a different standpoint, and as we have in some respects amplified their observations, and in others have obtained results not quite in accord with theirs, we feel justified in here recording our experiments.

It was observed by E. Salkowski in 1888 that blood could not be preserved by adding chloroform, because it gradually became converted into a thick mass.

In 1891 it was observed by Horbaczewski† that hæmoglobin was precipitated from a solution containing it, and kept at a temperature of 40—50° C., to which chloroform was added as a preservative.

The subject was investigated more minutely by Formánek‡ in 1900, and this observer found that a solution of hæmoglobin kept at 50—55° C. for some time with chloroform was completely precipitated, the filtrate being entirely free from hæmoglobin.

Formánek dried and analysed the precipitate, and from the absence of chlorine after fusion with sodium carbonate and potassium nitrate came to the conclusion that the precipitate is not a chloroform compound of hæmoglobin. In our opinion this is not a valid proof, as the chloroform need not be so stably combined with the chloroform as to stand drying at 130° C., and subsequent fusion as employed by Formánek. The precipitate was dissolved by Formánek after thorough washing to remove the chloroform by the addition of a few drops of sodium carbonate solution, and the solution gave the bands of oxy-hæmoglobin, and on treatment with ammonium sulphide reduced hæmoglobin. Formánek also found that blood serum and egg-albumin were precipitated (when the reaction of the fluid was acid or neutral) on keeping at a temperature of 50—55° C. in presence of chloroform. From these experiments this observer came to the conclusion that the precipitate with which he was dealing was a mixture of hæmoglobin and other proteids thrown out of solution by the chloroform. It is also stated in this paper that oxy-hæmoglobin is only slowly and incompletely precipitated by the action of chloroform at room temperatures.

E. Salkowski, in his later paper,§ states that blood kept at a temperature of 40° C. for 24—48 hours in presence of chloroform changes to a thick mass, but found that the precipitation of the hæmoglobin was not complete under such circumstances.

Regarding the action of chloroform on serum, he states that serum

* 'Zeitsch. f. physiol. Chem.,' vol. 29, 1900, p. 416.
† Quoted by Formánek, *loc. cit.*
‡ 'Zeitsch. f. physiol. Chem.,' vol. 29, 1900, p. 416.
§ 'Zeitsch. f. physiol. Chem.,' vol. 31, 1900, p. 329.

can be preserved in contact with chloroform for years without precipitation at room temperatures, and finds this in agreement with Formánek's results, who found, in presence of an alkaline reaction, no precipitation of serum by chloroform even at a temperature of 50—55° C. Formánek does not state whether his alkaline reaction is the natural alkaline reaction of the serum. Salkowski also found a precipitating action of chloroform upon solutions of albumose and casein.

Our experiments were conducted with hæmoglobin and serum obtained from pig's blood. The serum used was obtained from clotted blood and was thoroughly centrifugalised before use. The hæmoglobin was in all cases obtained by centrifugalising the blood corpuscles three times with normal saline, and then laking with distilled water and making up to the same volume as that of the blood taken.

In the case of serum we found that the fluid acquired, with less than 1 per cent. of chloroform (and greater quantities up to saturation), a peculiar opalescent and fluorescent appearance, but remained quite transparent to transmitted light. On the addition of over 2 per cent. of chloroform, there is a tendency to precipitation even in the cold, and at the end of 24—48 hours there is a slight precipitate present, but the effect is much hastened on placing the mixture in an incubator at 40° C., so that it becomes impossible to determine the maximum solubility of chloroform in serum at body temperature. Both in obtaining precipitation in the cold and more rapidly at 40° C. in presence of the natural alkaline reaction of the fluid, our results are at variance with those of Formánek and Salkowski. The results were obtained several times in succession.

The marked opalescence in the serum was obtained in preparation of solutions of known concentration in chloroform for purposes of measurement of their vapour pressures, and led us to doubt at first whether we were not dealing with a fine emulsion of chloroform in the serum. Since this point was of vital importance to our experiments on vapour-pressure, we investigated it as completely as possible.

In the first place, examination with the microscope of the opalescent fluid showed no visible globules of chloroform, even with the highest powers.

To make certain of the matter, a current of air from an aspirator was bubbled first through chloroform contained in a Woulff's bottle, afterwards through a similar bottle containing water, and then, *at the same temperature*, was sent through a third Woulff's bottle containing serum. By this procedure the serum never came in contact with fluid chloroform, nor with air more highly charged with chloroform vapour than corresponded to the saturation of the air in contact with it or passed through it.

There could, hence, be no condensation of chloroform and no means

by which an emulsion of chloroform, finer even than could be seen with a microscope, could be formed.

Very soon, however, after the chloroform vapour began to pass through, a distinct difference in appearance was observable between the serum and a control placed alongside, and after a time the serum charged with chloroform in this manner was as opalescent as the specimens made in the usual way by shaking with weighed quantities of chloroform, and gave similar results with regard to vapour pressure.

These results show that the marked opalescence is not due to an emulsion of chloroform, and further that it is not due to precipitation of proteid in the ordinary sense of the word, for no precipitate can be seen with the microscope in the opalescent fluid.

Further, experiments on the vapour pressure show that the value of this is a long way from the maximum value, which it must obviously possess if an emulsion was present.

A similar opalescence is obtained on adding to serum other organic liquids which possess anæsthetic properties, thus we have obtained it on saturation of serum with ether,* benzol and xylol, but have not followed the matter up as we have done with chloroform.

In the case of hæmoglobin solutions, we have not been able to observe an opalescence, similar to that seen in the case of serum, up to the strength at which precipitation begins. In order to study where precipitation commences it is necessary to keep the hæmoglobin and chloroform constantly stirred, or otherwise before the chloroform has dissolved the lower layer of hæmoglobin solution in contact with the liquid chloroform becomes precipitated. When precautions are taken to prevent this occurring, precipitation is found to take place when about 2 per cent. of chloroform has been added, that, is long before saturation is reached (*vide infra*). This precipitation prevents both the determination of the solubility of chloroform in hæmoglobin solutions, and the observation of the vapour pressure with high concentrations. With concentrations of 1 per cent. or under no precipitation whatever was found to occur. Contrary to Salkowski and Formánek, we found that no raising of the temperature was required to cause precipitation of the hæmoglobin; in fact, with strong solutions, the precipitation occurs within a few hours, even in the ice chamber. With time the precipitation is complete, and the precipitate is insoluble in water or saline, but in dilute sodium carbonate it is easily soluble, and we have found that the solution then shows the spectrum of alkaline hæmatin and not that of oxy-hæmoglobin as was found by Formánek.

* The opalescence with ether is not nearly so well marked, but increases on standing, and the solution in time becomes extremely viscid.

II.—*On the Relative Solubility of Chloroform in Water, Saline, Serum, and Hæmoglobin Solution.*

In so far as we have been able to discover, no attention has been paid by previous experimenters to the maximum amount of chloroform capable of solution in the blood or serum as compared with that taken up by water or saline solution isotonic with blood.

When any reference is made to the matter it has been usually assumed on general principles that the serum or plasma will behave like a saline solution of equal concentration and dissolve somewhat less chloroform than water.* In other words, that there is no specific action of the proteids or other substances in the plasma.

This supposition we have found experimentally to be entirely erroneous, for both serum and solutions of hæmoglobin dissolve much more chloroform than water or normal saline solution. This fact is of importance in regard to the mode of action, as it definitely points to an interaction between the chloroform and the proteid present.

The presence of fats would of course increase the apparent solubility of chloroform in serum, and hence it is necessary in all cases to use perfectly clear serum, free from suspended fat; this precaution we have always been careful to observe, and in addition the serum has always been centrifugalised.

In this connection it may be added that the hæmoglobin solutions which we have employed could not contain fatty matter, and hence the high solubilities which we have observed could only arise from chemical interaction between the hæmoglobin and the chloroform.

Methods for Determining Maximum Solubility.

Three methods have been used in the determination of the maximum solubility of chloroform in the solvents mentioned above, which have given concordant results and shown that the solubility in proteid solution is much higher than in water or saline.

In the first method we have determined the amount of chloroform dissolved by obtaining the product of volume and vapour pressure at low pressure and with a small volume of fluid, so that practically all the chloroform was simply pumped off into the vacuum. In this method the volume of fluid experimented with is necessarily small, and this gives rise to experimental error of measurement, which is added to by the volume measured being large and pressure small, so that the results are only approximative, yet it is observable that they confirm those obtained by the more accurate methods described below.

The details of the method are described in the succeeding section on the relationship between vapour pressure and concentration of

* Overton, 'Studien über die Narkose,' p. 93.

chloroform, and it need only be mentioned here that we have obtained solubilities of 0·95 per cent., in normal saline (0·75 per cent.), 3·33 per cent. in serum and 4·42 per cent. in whipped blood, by this method.

The second method employed consists in weighing out known amounts of chloroform into water and serum and hæmoglobin solution respectively, and then determining by direct observation that concentration in each case at which the chloroform ceased to be dissolved.

This method of observation is made easy in the case of chloroform by the high specific gravity of that fluid, as a result of which on inverting the flask in which the determination is being made even minute globules of undissolved chloroform can be seen falling through the fluid.

The determinations of solubility by this method are made on the following plan. Pure chloroform is dropped from a fine capillary pipette into a tared graduated flask of 25, 50 or 100 c.c., and carefully weighed to definite amount, corresponding, when the flask has been filled by the solvent under experiment, to a definite percentage of chloroform. A series of such flasks is prepared, and immediately after each flask is filled with the desired solvent and either shaken thoroughly by hand until solution is complete, or placed on a rotary shaking machine. After the lapse of several days, during which time the flasks are never opened and are kept shaken up, it is noticed at what level of concentration the chloroform ceases to be completely dissolved, and so the solubility is determined.

The lower strengths of known value short of saturation, and also the saturated solutions so prepared, were kept and used also for the experiments on vapour pressure at varying concentration described in the next section of this paper.

The following results were obtained by the application of this method at room temperatures, approximately (13° C.), for the percentage by weight dissolved:—

Water, 0·8 per cent. dissolved, 0·9 per cent. dissolved, 1 per cent. not dissolved completely. Estimated solubility 0·95 per cent.

Saline Solution (0·75 per cent. sodium chloride in water), 0·7 per cent. dissolved, 0·8 per cent. dissolved, 0·9 per cent. not dissolved. Estimated solubility about 0·83 per cent.

Serum, 3 per cent. dissolved, 3·5 per cent. dissolved, 4 per cent. all dissolved save a few small globules.

Hæmoglobin Solution or Blood.—Over 6 per cent. by weight is taken up when chloroform is shaken with blood or hæmoglobin solution of equal strength to the blood, prepared from blood by centrifugalising several times with saline, and subsequent laking with distilled water, and no globules of chloroform can be seen on careful examination with the microscope. But the solution rapidly changes in colour, and a

precipitate is thrown out on standing, as above described, which is quite insoluble in water or saline, but easily soluble in dilute sodium carbonate solution, and then gives the spectrum of alkaline hæmatin.

The blood begins to give this precipitate when about 1·5 per cent. of chloroform has been added at room temperature, but with a lower concentration, and more rapidly when heated to body temperature in the incubator. Two per cent. of chloroform gives a precipitate in the cold, and on heating to 40° C. a red flocculent precipitate leaving a clear colourless fluid above.

The third method for determining the solubility of chloroform in the fluids experimented with consists in shaking up thoroughly for several hours with an excess of chloroform, and then pipetting off and determining the amount of chloroform in the solution.

The difficulty here is a rapid and accurate method of determining the amount of chloroform contained in a measured volume of the given saturated solution.

The procedure finally employed for this purpose, which is also being experimented with as a method for quantitative estimation of chloroform in blood and serum at lower values reaching to the anæsthetising value, was as follows, and led to very accurate results.

A measured volume (usually 10 c.c.) of the fluid saturated with chloroform is placed in a flask fitted airtight with a double bored cork, and a stream of hydrogen is aspirated through the solution, the oxygen present in the flask and connections is absorbed by passing through alkaline pyrogallate, and the mixture of hydrogen and chloroform is then burnt by passing over heated palladium asbestos placed in a very short combustion tube. All the chlorine in the chloroform is thus burnt to hydrochloric acid, and the amount of this absorbed in standard alkali is then estimated, first by back titration against standard acid, and then further checked, either by volumetric titration with standard silver nitrate solution, or by gravimetric determination as silver chloride.

The serum used in these determinations was examined for chloroform emulsion by the microscope, but no undissolved chloroform in suspension was observed. The precipitate in serum at atmospheric temperature obtained by this method of shaking up with excess of chloroform was very dense, so that the serum became quite opaque.

The results obtained by employing this method were as follows :—

Distilled water, dissolved 0·95 per cent., and serum, dissolved 5·08 per cent.

III.—*On the Vapour Pressure of Chloroform Dissolved in Varying Concentration in Water, Saline, Serum, and Hæmoglobin Solutions respectively.*

A determination of the vapour pressure of an anæsthetic in solution at varying concentrations in serum, in hæmoglobin, or in blood, is of

high practical importance, since it is upon the relationship of this vapour pressure to the concentration of the solution that the amount of anæsthetic taken up by the blood circulating through the lungs depends.

It has hitherto been taken for granted that the Dalton-Henry law can be applied, and that the amount of anæsthetic taken up is strictly proportional to, and varies directly with, the percentage of the vapour of the anæsthetic in the inspired air.

This has never, however, to our knowledge, been experimentally tested, and it seemed to us desirable to attempt such a determination. We have investigated from this point of view solutions of chloroform in serum, hæmoglobin solution (of equal strength in hæmoglobin to the blood from which the hæmoglobin was prepared) and whipped blood, and have contrasted the pressures obtained with those of solutions in chloroform, in water, and normal saline at equal concentrations.

The vapour pressures have been measured corresponding to concentrations ranging from considerably below the anæsthetising values for chloroform vapour pressure in air (viz., 8—10 mm.) observed by Paul Bert, up to the saturation points in most cases.

Apparatus.

The instrument employed for this purpose was a form of "differential densimeter," which, after passing through many modifications, took the form represented in the accompanying sketch (fig. 1), which is drawn approximately to a scale of $\frac{1}{4}$.

The two tubes shown are exactly similar, and are graduated in cubic centimetres and tenths in the upper portion, and in centimetres in the lower and wider portion.

The tubes are connected as shown by means of thick-walled rubber tubing and a glass Y-piece to a stout glass mercury receiver capable of holding more than enough mercury to fill both tubes and their connections.

The tubes are held in a vertical position by clamps attached to the strong vertical iron bar of a massive retort stand, and each tube is capable of being moved in its clamp vertically up and down for purposes of adjusting the mercury levels.

In order to keep a constant temperature (in the case of the experiments carried out at body temperature) the upper portion of each tube, from about the middle of the wide part to the level of the stopper at the top, was encased in a hot-water jacket of the form shown in detail in fig. 2, and omitted for clearness from fig. 1.

It was found convenient to use for the outer glass tubing of this jacket the largest size of a common variety of paraffin-lamp chimney,

which measured 8 cm. in diameter above, bulged out as shown to 9 cm., and then narrowed below to 6 cm.

The wider top and bulge were found very useful in facilitating the introduction and vigorous movements of the bar electro-magnet used as

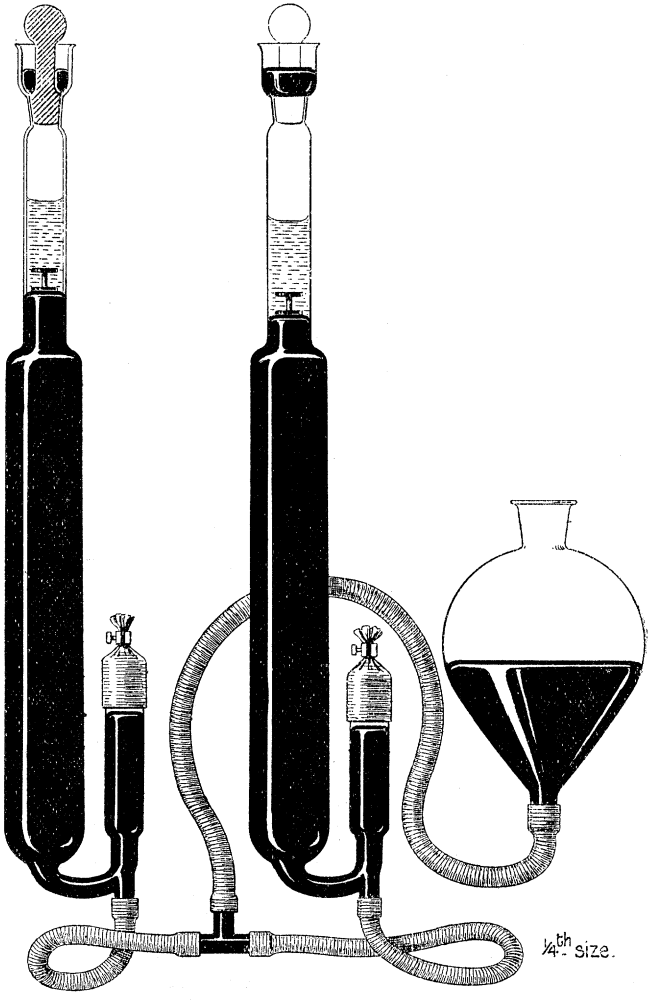


FIG. 1.—Diagram of differential Densimeter.

a stirrer in connection with the iron stud (seen in figs. 1 and 2, at the top of the mercury), which was dropped into each tube, and, during an experiment, agitated up and down so as to thoroughly mix the fluid under experiment, and so bring it into equilibrium more rapidly with the vapour in the space above it.

The hot-water jacket was made watertight below by means of an india-rubber cork, as shown (fig. 2); the rubber cork was also bored in each case for two narrow glass tubes, for the purpose of carrying

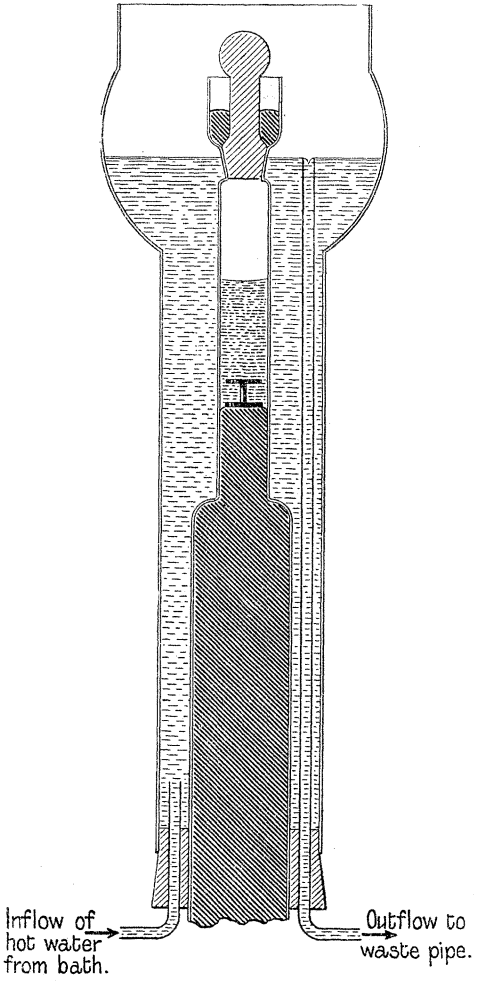


FIG. 2.—Section taken through the upper portion of one tube of the differential Densimeter and the hot-water jacket, showing the inflow and outflow tubes. Scale $\frac{1}{3}$.

water to and from the jacket. The two tubes carrying the water in, stopped about 2 cm. above the upper surface of the rubber cork in each tube, so as to prevent blocking by mercury accidentally run over from the top when the inner tubes were being cleared out at the termination of a measurement. The outer ends of these two tubes

were attached by means of narrow rubber tubings and a glass Y-tube to a bath of hot water, placed at a higher level, and a screw-down clip on each rubber tube regulated the flow until a thermometer placed in the corresponding hot-water jacket showed the desired temperature. A constant level of water was kept up automatically in the warm supply bath, and its temperature was regulated so as to lie 2—3° above that of the jackets. The two outflow tubes passed up, as shown, inside the jacket to the level of the ground in glass stopper, and their outside ends were connected by means of rubber tubing to the waste pipe.

In fig. 1 the upper portion of the left-hand tube is shown in section, and that of the right-hand one in outline. The ground in stoppers shown were found, when sealed with mercury, to be much more effectual against minute leakages, which entirely vitiate the results, than any form of tap, and they are also much more convenient for introducing the solutions to be experimented upon, and for cleaning out the apparatus. Further, since they do not require to be operated between the commencement and termination of each determination of vapour pressure, they are better adapted to their particular purpose than a tap. In the course of our experiments, we also found it necessary to be able to dilute a solution with more of its solvent without allowing it to come in contact with any appreciable volume of air, and for this purpose found the stopper arrangement most convenient.

The side tube shown at the lower end of each main tube was designed to trap air which was found to slowly leak in through the rubber pressure tubing, when a vacuum was established by lowering the mercury receiver, and for a long time was a source of annoyance. We subsequently learned that the device had been first introduced by Lord Rayleigh. At the end of an experiment, any air which has collected is discharged by raising the mercury-holder, opening the screw-clip shown, and allowing enough mercury also to pass through to form a seal in the rubber tubing above the clip.

The mercury-holder was suspended by means of a ring and loop of wire attached around the bulb from a vertical rod, swivel, and hook, possessing a slow screw movement in a block attached to a horizontal rod fixed in a clamp, which could be moved up and down on a heavy retort stand. For large movements, the clamp was slid up or down the retort standard, and for fine movements the screw was raised or lowered in its block.

In using the apparatus, the two vertical tubes are first placed at the same level, the mercury-holder is filled with mercury, and, with the two glass stoppers out, the whole apparatus is filled with mercury, the two stoppers are next inserted, enough mercury being left above them to form a seal, and the mercury-holder is then lowered until the two vertical tubes become evacuated. The receiver is then raised

again to the level of the stoppers, and any bubble of air found is discharged.

The apparatus is now ready for an experiment, and, with stoppers out, the levels of mercury are adjusted until there is an equal volume left above the mercury on each side. A given volume of the solvent (say 5 c.c.) is now introduced on the one side (say left), and an equal volume of the solution of chloroform in the same solvent on the other side. In each case, immediately after the fluid has been introduced, the stopper is inserted, care being taken to prevent any air being included, either as a bubble at the mercury surface, or between the surface of the introduced fluid and the stopper. To achieve the latter end, we have almost always introduced above the mercury 2 or 3 c.c. more than the required quantity, so that it stood in the neck and slightly above, and then, by easing the stopper and gently adjusting the level of the mercury-holder, have brought the level of the mercury in the tube to the desired volume mark. After the solvent on the one side, and the solution of chloroform of the desired strength on the other side, have been successfully introduced in equal volume, and without any bubble of air, the mercury-holder is lowered until a space containing vapour has appeared on each side. The level of the mercury will be found to be lower on the chloroform side, and it is obvious, the instrument being independent of variations in atmospheric pressure, and the only different factor being the added chloroform on the one side,* that the difference in pressure will give the vapour pressure directly for that strength of chloroform solution at that particular temperature.

There is hence no need to determine pressure due to dissolved gases on the two sides,† or pressure of aqueous vapour, since these balance, and the quickness with which readings can be directly obtained makes it possible to carry out a long series of determinations at varying strengths, without the proteid solutions having time to undergo bacterial change.

Certain precautions have to be taken, however, and corrections made which may here be mentioned:—

1. Before taking a reading it is essential to move the tubes vertically and adjust the levels until the volumes of the vapour spaces above the upper aqueous solution meniscus in each case are exactly equal, otherwise inequality in pressure of gases pumped off on the two sides gives rise to an error, which is greater the smaller the vapour space.

* There will be a small difference in the pressure of water vapour on the two sides, due to there being a stronger solution on the chloroform side, but this is in all cases too minute compared to the pressure of the chloroform vapour to make any appreciable error.

† Slight differences in dissolved gases gave a disturbance with very dilute chloroform solutions, and this was later obviated by pumping the gases off (*vide infra*).

2. Before taking a reading, there must be certainty that each fluid is in equilibrium with its vapour space. This is shown by absence of variation when the apparatus is left at rest.

For rapid and accurate working the mechanical stirring by means of the studs and magnet is indispensible, for even after the lapse of an hour when at rest the solution has not completely discharged its proper amount of chloroform into the vapour space. When once the control, containing, of course, no chloroform, has been thoroughly stirred it remains constant, and need not be changed at the end of each determination, but can be used throughout an entire experiment.

By vigorous stirring, equilibrium can be attained in 5—10 minutes, and the level does not afterwards change no matter how long stirring and observation be kept up. This important experimental observation we have taken occasion to verify several times during our experiments.

3. For very accurate working, especially with the dilute solutions and low pressures, it is necessary in the case of serum and hæmoglobin to pump off the dissolved gases by means of a Töpler pump, otherwise these come off unequally from solvent and solution and disturb the results at the low pressures. The chloroform solutions are then made up from the pumped-out solvent, which also must be used for control and for making the dilutions.

4. The temperature must be the same in the jackets surrounding each tube at the time when each reading is taken, and in a series of determinations at varying strength and a constant temperature, that temperature must be closely maintained throughout. The temperature error is a maximum when the solutions are near saturation, for then the variation in vapour pressure per degree is very large; fortunately here the differences in level under observation are also very large, which diminishes the percentage error arising from small deviations in temperature.

At concentrations away from saturation, the variations arising from small differences in temperature approximately obey the gas law, and under the conditions of our experiments become quite negligible.

5. A correction must be made in all cases, upon the concentration of the solution introduced into the tube, for the amount of chloroform pumped off from the solution into the vapour space. This correction is, of course, larger in the case of the more concentrated solutions with high vapour pressures.

This has been done in the experiments of which records are given below, and accounts for the concentrations not being exact percentages or small fractions of exact percentages.

The amount of chloroform in the vapour space is readily calculated from the product of the observed vapour pressure and the volume of the vapour space, and this amount deducted from the quantity contained in the chloroform solution when it was introduced, gives the necessary

datum for calculating the concentration in chloroform of the solution corresponding to the observed vapour pressure in the vapour space.

The ratio of the vapour concentration in the fluid to the concentration in the vapour space gives the coefficient of distribution (*coefficient de partage, Theilungscoefficient*): this should remain constant if the absorption of the chloroform vapour by the liquid were normal and strictly proportional to the vapour pressure, and if it varies it points to a physical or chemical aggregation or compound between the chloroform and the fluid or its constituents (*vide infra*).

Method of Reading.—The readings were taken with a cathetometer,* placed about 4 feet from the tubes, both for greater accuracy in reading than direct measurement would give and to avoid changes in temperature.

Two Methods of Experimentation.—We have employed two different methods of experimentation in investigating the variation in vapour pressure with varying concentration. It is obvious that the concentration of a measured volume of a strong solution introduced into the densimeter may be diminished by pumping off more and more chloroform, by increasing the volume of the vapour space above the solution.

A series of readings of differences in pressure may thus be obtained in which the vapour space on the two sides is kept equal and of known and increasing value throughout the series. This method we have called the method of "*variable vapour space.*"

On the other hand, a series of solutions of known and steadily diminishing or increasing concentration may be introduced into the densimeter and measured one after another as to their vapour pressure, in each case with a known fixed volume of vapour space. As stated above, the concentration at which each vapour pressure in the series is measured is then accurately known. This method we have called the method of "*constant vapour space.*"

Method of "Variable Vapour Space."—In this method, unless the volume of the tubes of the densimeter is very large, the volume of solution and solvent respectively introduced must be very small. We have usually taken $\frac{1}{2}$ c.c. on each side, either of a saturated solution or of a very strong solution of known strength, and by altering the levels of the tubes and mercury receiver, have taken a long series of readings, in each case with equal volume of vapour space on each side, at every increasing volume of vapour space, until the difference in pressure became of small value, and the product of volume and vapour pressure became approximately constant, showing that practically all

* The instrument used was made by Pye and Co., of Cambridge, and, by means of a vernier and divided screw-head, read to $\frac{1}{100}$ of a millimetre. We have frequently observed that we were able to take readings within five divisions, that is $\frac{1}{20}$ mm., which is far within the accuracy of other portions of our determinations.

the chloroform had been pumped off from the solution. This constant product then gave the necessary datum for calculating the concentration of the original solution introduced into the densimeter, the product of vapour pressure and volume at each stage gave the datum for calculating the quantity of chloroform pumped off from the solution, and therefore for deducing the corresponding concentration of solution. Or, also, by plotting vapour pressures as abscissæ, and the product of vapour pressure and volume of vapour space as ordinates, the ratio of vapour pressure and amount of chloroform absorbed at each stage could be shown.

The method of "*variable vapour space*" has, however, two working disadvantages, which caused us in the end to abandon it and replace it by the method of "*constant vapour space.*" The first objection is that the amount of solution taken is small, hence it is difficult to measure it with accuracy, and to make it equal on the two sides.

The second objection is that at the low concentrations the increase of volume for a small fall in pressure is very large, and hence the determinations become inaccurate, a small error in pressure reading making a large deviation. The values for high pressures are also inaccurate, but for a different reason; these readings are taken with small volumes of vapour space, and unless the vapour spaces are accurately equal on the two sides, there is a large disturbance due to inequality in pressure of the previously dissolved gases pumped off on the two sides.

The results, however in the intermediate pressures are accurate and are given below, as they confirm those given by the other method.

Method of "Constant Vapour Space."—In using this method we have always introduced a volume of 5 c.c. of the solvent on one side, and 5 c.c. of a solution of known strength on the other, and have invariably adjusted the levels so that at the temperatures of observation there was a vapour space of exactly 5 c.c. on each side.

In some cases we have started with a saturated solution of chloroform and have then made dilutions of different percentages of that solution, in the manner described below. In the later experiments we found it, however, more expedient, on account of knowing the exact concentration directly, to prepare a solution of known strength, say 1 per cent., and for the more dilute solutions to use various percentage dilutions of this stock solution. The more concentrated solutions were obtained by making up solutions varying by 1 per cent. in strength, and $\frac{1}{2}$ per cent. differences were got by mixing these with each other in equal proportions. For percentages less than 0·1 per cent., a 0·1-per-cent. solution was first prepared by making a ten-fold dilution of the 1-per-cent. solution, and this 0·1-per-cent. solution was then diluted similarly to the 1-per-cent. solution.

Precautions in Preparing, Preserving, in Unaltered Strength, and Diluting by known Amounts, of the Solutions Used.

In working with the solutions, it is indispensable that due precautions be taken against loss by escape of chloroform into the air during the various manipulations.

If a portion, for example, of a stock solution of known strength be pipetted off for use in the densimeter, and then the stock bottle be merely stoppered, the air space over the portion of solution left in the bottle rapidly becomes charged with chloroform vapour at the expense of the stock solution, and a second sample taken later from the same bottle will be found to be weak and give a wrong result. The way to guard against this is to fill the bottle with mercury up to the neck immediately after drawing off, and at once stopper up. Similar devices were employed in all dilutions to complete the process out of contact with air.

The stock solutions were made by direct weighing, by dropping from a fine pipette, into graduated glass-stoppered flasks of 25, 50, or 100 c.c. capacity, according to the amount required, immediately filling up to the mark with the particular solvent, and setting at once upon a slowly rotating disc, driven at such a rate that the chloroform globules have just time to fall through the solution each time the flask is inverted. In this way, a rapid solution is effected, so saving much time. Further, in certain cases such shaking is indispensable, for a solution of hæmoglobin left in contact with even a small amount of chloroform without continuous shaking soon forms a precipitated layer at the bottom.

The stock solutions were kept carefully stoppered, unless at the moment of introducing the pipette to remove a portion for experiment, and then the space within the bottle was always filled to the stopper with mercury in the manner above described.

We found much difficulty owing to leakage of chloroform during dilution in the case of the strengths intermediate between the stock strengths, until it occurred to us to make these dilutions in the densimeter itself. For this purpose we always placed the mercury level at 10 c.c. below the stopper, on the side of the densimeter in which the mixing was to be carried out, then the proper amounts of the two solutions necessary to give the desired strength was drawn up in two graduated pipettes and run into the densimeter tube, the stopper was then inserted, including only a minute bubble of air, and now by thorough agitation of the iron stud through the fluid by means of the electro-magnet a thorough mixing of the two fluids was attained, then by raising the mercury-holder and slightly easing the stopper 5 c.c. were allowed to escape, the mercury level being placed accurately at 5 c.c. A seal of mercury was finally dropped in above the stopper, and so the dilution was effected.

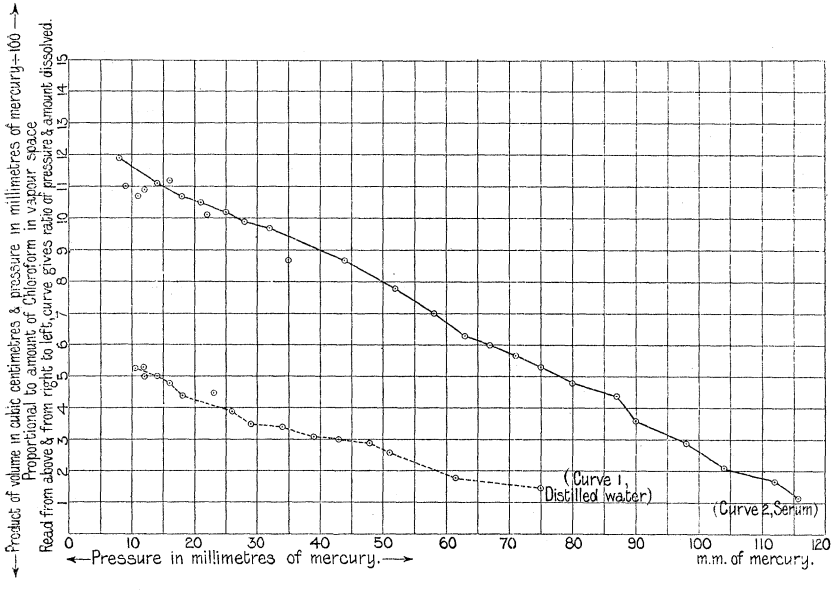
For example, to obtain a dilution of 1·5 per cent., 5 c.c. of 1-per-cent. solution and 5 c.c. of 2-per-cent. solution were drawn off into pipettes, placed in the densimeter and mixed as above described; to obtain a 0·4-per-cent. solution, 6 c.c. of the solvent were taken and 4 c.c. of a 1-per-cent solution, and similarly treated; to obtain a 0·03-per-cent. solution, 7 c.c. of solvent and 3 c.c. of 0·1-per-cent. solution were taken, and so on.

Certain of our experiments were carried out at room temperature and others approximately at body temperature (40° C.); the following protocols and accompanying curves show some of the typical results obtained, which have been confirmed in most cases by duplicates :—

Variable Vapour Space.

Experiment 1.—Distilled water containing approximately 0·78 per cent. of chloroform. Half a cubic centimetre was introduced into each tube of the densimeter, of the chloroform water on one side and of the same distilled water without chloroform on the other. The temperature at which the experiment was carried out was 17° C., and the volume at which readings of pressure were taken varied from 2—50 c.c. The percentage of chloroform in the water was not known directly, but was calculated by extrapolation of the curve showing V.P., see Curve 1, fig. 3.

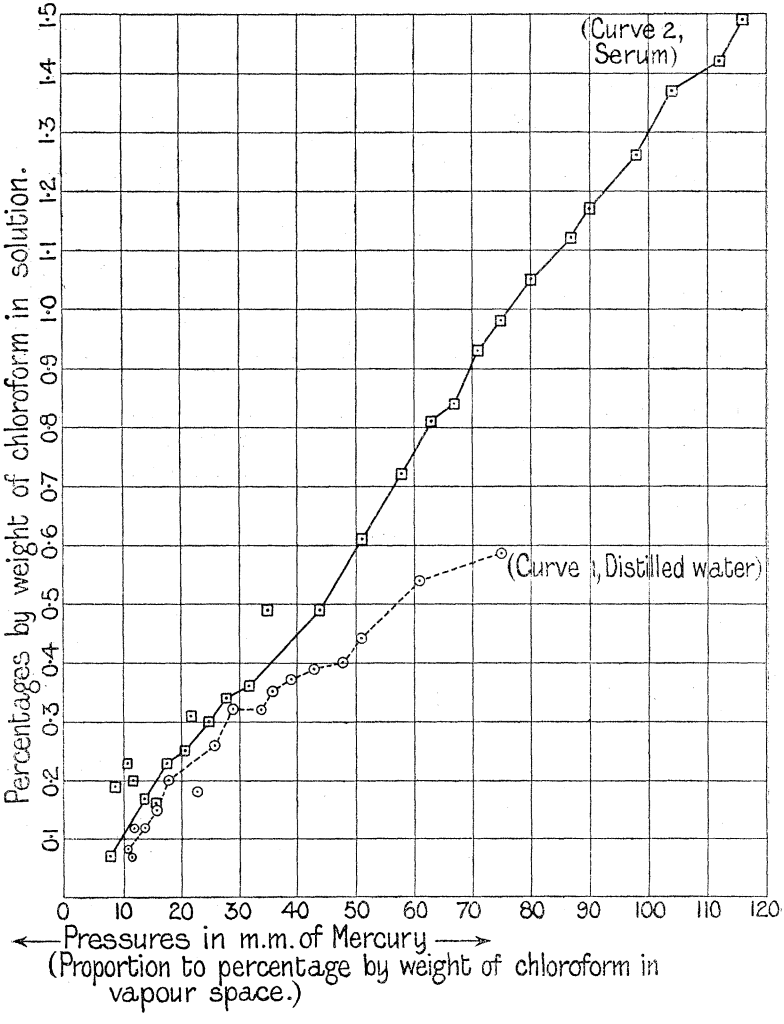
FIG. 3.



The following table gives the results of the experiment, which are also shown graphically in Curve 1, fig. 3, in which the abscissæ show

pressures of chloroform vapour and the ordinates the product of the volume and pressure of vapour. In Curve 1, fig. 4, the same experi-

Fig. 4.



ment is shown with pressures of chloroform vapour as abscissæ, and percentage of chloroform dissolved at each pressure as ordinates:—

Experiment 1.—Distilled Water.

Temperature 17° C.

Volume of vapour space.	Pressure of chloroform in vapour space in mm. of mercury.	Percentage by weight of chloroform in vapour space.	Weight in grammes of chloroform in vapour space.	Weight in grammes of chloroform in solvent.	Percentage by weight of chloroform in solvent.	Coefficient of distribution between vapour space and solvent.
2	74·61	0·04957	0·00099	0·00293	0·586	1 : 11·8
3	60·62	0·04027	0·00121	0·00271	0·542	1 : 13·4
5	51·29	0·03408	0·00170	0·00222	0.444	1 : 13·0
6	47·69	0·03168	0·00190	0·00202	0·404	1 : 12·7
7	43·08	0·02862	0·00197	0·00195	0·390	1 : 13·6
8	38·91	0·02585	0·00207	0·00185	0·370	1 : 14·3
9	35·98	0·02390	0·00215	0·00177	0·354	1 : 14·8
10	34·36	0·02283	0·00228	0·00164	0·328	1 : 14·4
12	28·83	0·01915	0·00230	0·00162	0·324	1 : 16·9
15	26·16	0·01738	0·00263	0·00129	0·258	1 : 14·8
20	22·62	0·01503	0·00301	0·00091	0·182	1 : 12·1
25	17·65	0·01173	0·00293	0·00099	0·198	1 : 16·9
30	15·84	0·01052	0·00316	0·00076	0·152	1 : 14·5
35	14·37	0·00954	0·00334	0·00058	0·116	1 : 12·2
40	12·43	0·00835	0·00334	0·00058	0·116	1 : 13·9
45	11·87	0·00787	0·00354	0·00038	0·076	1 : 9·7
50	10·54	0·00700	0·00350	0·00042	0·084	1 : 12·0

Experiment 2.—Serum containing approximately 1·65 per cent. of chloroform, the amount being determined by extrapolation of Curve 2, fig. 3. Experiment conducted in all respects similarly to Experiment 1. Graphic records of results shown by Curve 2, in figs. 3 and 4.

Experiment 2.—Serum.

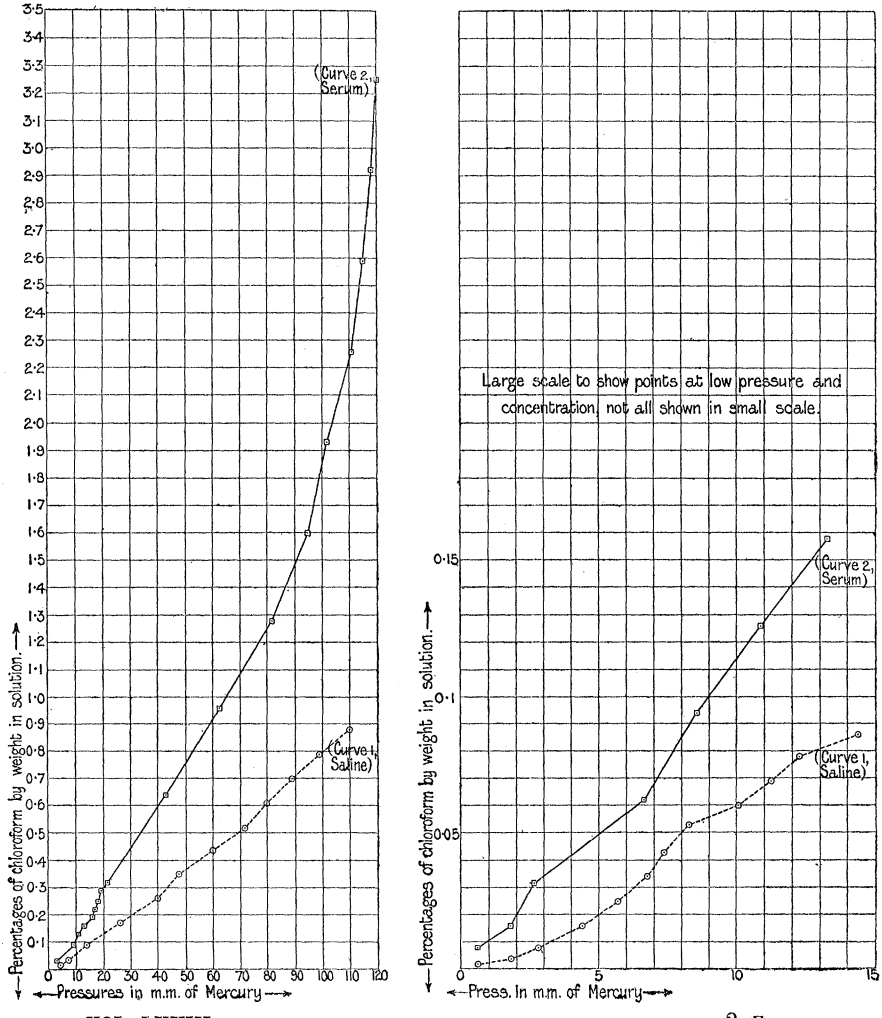
Temperature 18° C.

Volume of vapour space.	Pressure of chloroform in vapour space in mm. of mercury.	Percentage by weight of chloroform in vapour space.	Weight in grammes of chloroform in vapour space.	Weight in grammes of chloroform in solvent.	Percentage by weight of chloroform in solvent.	Coefficient of distribution between vapour space and solvent.
1	115·63	0·07655	0·00077	0·00746	1·492	1 : 19·5
1·5	111·85	0·07405	0·00111	0·00712	1·424	1 : 19·2
2	104·27	0·06904	0·00138	0·00685	1·370	1 : 19·9
3	98·15	0·06498	0·00195	0·00628	1·256	1 : 19·3
4	89·89	0 05763	0·00237	0·00586	1·172	1 : 20·4
5	87·05	0·05277	0·00264	0·00559	1·118	1 : 21·2
6	79·70	0·04975	0·00299	0·00524	1·048	1 : 21·7
7	75·14	0·04730	0·00331	0·00492	0·984	1 : 20·6
8	71·46	0 04487	0·00359	0·00464	0·928	1 : 20·7
9	67·16	0 04417	0·00402	0·00421	0·842	1 : 18·9
10	63·24	0·04187	0·00419	0·00404	0·808	1 : 19·3
12	58·05	0·03843	0·00461	0·00362	0·724	1 : 18·9
15	52·31	0·03463	0·00519	0 00304	0·608	1 : 17·6
20	43·64	0·02889	0·00577	0·00246	0·492	1 : 17·0
25	34·97	0·02315	0·00579	0·00244	0·488	1 : 21·1
30	32·38	0·02144	0·00643	0·00180	0·360	1 : 12·1
35	28·20	0·01867	0·00653	0·00170	0·340	1 : 18·2
40	25·44	0·01684	0·00674	0·00149	0·298	1 : 17·7
45	22·40	0·01483	0·00668	0·00155	0·310	1 : 20·9
50	21·03	0·01392	0·00696	0·00127	0·254	1 : 18·3
60	17·91	0·01186	0·00712	0·00111	0·222	1 : 19·0
70	16·03	0·01061	0·00743	0·00080	0·160	1 : 15·1
80	13·93	0·0092	0·00737	0·00086	0·172	1 : 18·7
90	12·12	0·0080	0·00722	0·00101	0·202	1 : 25·3
100	10·70	0·0071	0·00708	0·00115	0·230	1 : 32·4
125	8·77	0·0058	0·00726	0·00097	0·194	1 : 33·4
150	7·91	0·0052	0·00786	0·00037	0·074	1 : 14·3

Experiments with Constant Vapour Space.

Experiment 3.—Saline solution containing 0·75 per cent. of sodium chloride, and approximately 0·95 per cent. of chloroform, determined from product of pressure and volume after pumping off. Five c.c. of the saline introduced on one side as a control and 5 c.c. of the chloroform solution on the other, and a vapour space of 5 c.c. being formed on each side. Temperature of experiment 15° C. The results are shown graphically in Curve 1, fig. 5.

FIG. 5.



Experiment 3.—Saline.

Temperature 15° C.

Percentage of chloroform by weight in solution originally introduced.	Pressure of chloroform in vapour space in mm. of mercury.	Percentage by weight of chloroform pumped off into vapour space.	Percentage of chloroform by weight remaining in solution.	Coefficient of distribution between vapour space and solvent.
0·0024	0·56	0·0004	0·0020	1 : 5·0
0·0048	1·78	0·0012	0·0036	1 : 3·0
0·0095	2·76	0·0018	0·0077	1 : 4·3
0·0190	4·44	0·0030	0·0160	1 : 5·3
0·0286	5·65	0·0038	0·0248	1 : 6·5
0·0381	6·79	0·0045	0·0336	1 : 7·2
0·0476	7·38	0·0049	0·0427	1 : 8·7
0·0571	8·34	0·0056	0·0515	1 : 9·2
0·0666	10·14	0·0068	0·0598	1 : 8·8
0·0762	11·28	0·0075	0·0687	1 : 9·2
0·0857	12·25	0·0082	0·0775	1 : 9·5
0·0952	14·43	0·0096	0·0856	1 : 8·9
0·1904	25·68	0·0172	0·1732	1 : 10·1
0·2856	39·76	0·0256	0·2600	1 : 10·2
0·3808	47·96	0·0321	0·3487	1 : 10·9
0·4760	60·16	0·0402	0·4358	1 : 10·8
0·5712	72·21	0·0483	0·5299	1 : 10·8
0·6664	79·75	0·0534	0·6130	1 : 11·5
0·7616	88·67	0·0593	0·7023	1 : 11·8
0·8568	99·16	0·0663	0·7905	1 : 11·9
0·9520	110·39	0·0738	0·8782	1 : 11·9

Experiment 4.—Serum containing approximately 3·33 per cent. of chloroform determined as in Experiment 3. Experiment performed similarly to Experiment 3 and temperature also 15° C. Results plotted in Curve 2, fig. 5.

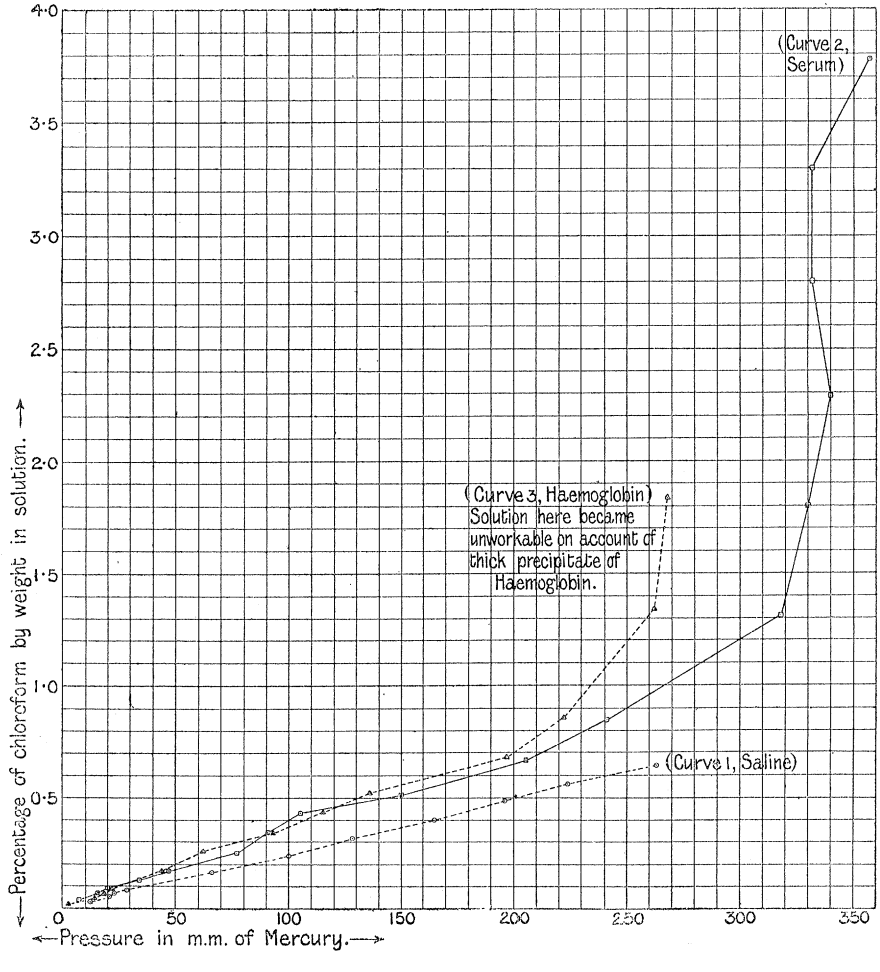
Experiment 4.—Serum.

Temperature 15° C.

Percentage by weight of chloroform originally introduced.	Pressure of chloroform in vapour space in mm. of mercury.	Percentage by weight of chloroform pumped off into vapour space.	Percentage by weight of chloroform remaining in solution.	Coefficient of distribution between vapour space and solvent.
0·0083	0·66	0·0004	0·0079	1 : 19·7
0·0167	1·82	0·0012	0·0155	1 : 12·8
0·0333	2·74	0·0018	0·0315	1 : 17·5
0·0666	6·66	0·0045	0·0621	1 : 13·8
0·0999	8·59	0·0057	0·0942	1 : 16·5
0·1332	10·85	0·0073	0·1259	1 : 17·3
0·1666	13·28	0·0089	0·1577	1 : 17·7
0·1999	15·54	0·0104	0·1895	1 : 18·2
0·2332	16·96	0·0113	0·2219	1 : 19·5
0·2665	18·37	0·0123	0·2543	1 : 20·7
0·2998	19·19	0·0128	0·2870	1 : 22·4
0·3331	22·49	0·0150	0·3181	1 : 21·2
0·6662	43·02	0·0281	0·6381	1 : 22·7
0·9993	63·15	0·0422	0·9571	1 : 22·7
1·3324	82·16	0·0550	1·2774	1 : 23·5
1·6655	95·30	0·0638	1·6017	1 : 25·1
1·9986	101·64	0·0680	1·9306	1 : 28·4
2·3317	110·53	0·0740	2·2577	1 : 30·5
2·6648	115·30	0·0771	2·5877	1 : 33·6
2·9979	117·53	0·0786	2·9123	1 : 37·1
3·3310	120·41	0·0810	3·2500	1 : 40·1

Experiment 5.—Saline containing 0·75 per cent. of sodium chloride, and the amounts of chloroform shown in the table, the maximum being 0·8 per cent. originally in solution. In this and succeeding experiments the amount of chloroform was known directly by weighing out as described above. The temperature in this and succeeding experiments was 40° C. The results of this experiment are shown in Curve 1, fig. 6.

FIG. 6.



Experiment 5.—Saline (0·75 per cent.).
Temperature 40° C.

Percentage by weight of chloroform originally introduced.	Pressure of chloroform in vapour space in mm. of mercury.	Percentage by weight of chloroform pumped off into vapour space.	Percentage by weight of chloroform remaining in solution.	Coefficient of distribution between vapour space and solvent.
0·04	11·75	0·0722	0·0328	1 : 4·6
0·06	20·59	0·0127	0·0473	1 : 3·8
0·08	23·29	0·0143	0·0657	1 : 4·6
0·1	29·35	0·0181	0·0819	1 : 4·5
0·2	65·62	0·0404	0·1596	1 : 4·0
0·3	99·83	0·0615	0·2385	1 : 3·9
0·4	129·11	0·0795	0·3205	1 : 4·0
0·5	166·04	0·1022	0·3978	1 : 3·9
0·6	196·05	0·1207	0·4793	1 : 4·0
0·7	224·04	0·1379	0·5621	1 : 4·1
0·8	262·52	0·1616	0·6384	1 : 4·0

Experiment 6.—Serum containing amounts of chloroform shown in table by direct weighing. Results shown in Curve 2, fig. 6.

Experiment 6.—Serum.
Temperature 40° C.

Percentage by weight of chloroform originally introduced.	Pressure of chloroform in vapour space in mm. of mercury.	Percentage by weight of chloroform pumped off into vapour space.	Percentage by weight of chloroform remaining in solution.	Coefficient of distribution between vapour space and solvent.
0·04	6·80	0·0042	0·0358	1 : 8·5
0·05	8·51	0·0052	0·0448	1 : 8·6
0·06	11·64	0·0072	0·0528	1 : 7·3
0·08	15·09	0·0093	0·0707	1 : 7·6
0·1	19·74	0·0122	0·0878	1 : 7·2
0·15	34·12	0·0210	0·1290	1 : 6·1
0·2	47·27	0·0291	0·1709	1 : 5·8
0·3	77·43	0·0477	0·2523	1 : 5·3
0·4	91·46	0·0563	0·3437	1 : 6·1
0·5	105·31	0·0663	0·4337	1 : 6·5
0·6	149·54	0·0921	0·5079	1 : 5·5
0·8	205·22	0·126	0·674	1 : 5·3
1·0	240·49	0·148	0·852	1 : 5·8
1·5	317·74	0·195	1·305	1 : 6·7
2·0	329·75	0·203	1·797	1 : 8·9
2·5	339·81	0·209	2·291	1 : 10·9
3·0	332·36	0·205	2·795	1 : 13·6
3·5	332·00	0·204	3·296	1 : 16·2
4·0	357·08	0·220	3·780	1 : 17·2

Experiment 7.—Solution of hæmoglobin, of equal strength in hæmoglobin to blood from which obtained, and containing the amounts of chloroform shown in the table. The results are shown graphically by Curve 3 of fig. 6.

Experiment 7.—Hæmoglobin Solution.

Temperature 40° C.

Percentage by weight of chloroform originally introduced.	Pressure of chloroform in vapour space in mm. of mercury.	Percentage by weight of chloroform pumped off into vapour space.	Percentage by weight of chloroform remaining in solution.	Coefficient of distribution between vapour space and solvent.
0·02	2·85	0·0018	0·0182	1 : 10·1
0·04	7·31	0·0045	0·0355	1 : 7·9
0·06	14·52	0·0089	0·0511	1 : 6·6
0·08	17·79	0·0110	0·0690	1 : 6·3
0·1	22·34	0·0138	0·0862	1 : 6·3
0·2	43·85	0·0270	0·1730	1 : 6·4
0·3	62·01	0·0382	0·2618	1 : 6·8
0·4	93·15	0·0573	0·3427	1 : 5·9
0·5	114·91	0·0707	0·4393	1 : 6·2
0·6	136·47	0·0840	0·5160	1 : 6·2
0·8	197·10	0·1213	0·6787	1 : 5·6
1·0	222·64	0·1371	0·8629	1 : 6·3
1·5	262·26	0·1614	1·3386	1 : 8·3
2·0	268·00	0·1650	1·8350	1 : 11·1

IV.—*Solubilities of Gases in Serum and Hæmoglobin in Presence of Chloroform.*

It was thought that such compounds as are shown by the above experiments to be formed between chloroform and serum or hæmoglobin solution, might interfere with the carriage of oxygen and carbon-dioxide by the blood. Accordingly, experiments were carried out upon serum and hæmoglobin solutions to test this point.

A volume of about 500 c.c. of serum or of hæmoglobin solution obtained as for the experiments in Section 3, was completely deprived of gases by exhaustion with a Töpler pump at 40° C., afterwards saturation with air or air and carbon-dioxide, was carried out upon two equal volumes contained in similar bottles to one of which a sufficient quantity of chloroform was added to make a 1-per-cent. solution, while the other served as a control. The gases dissolved in each case were then collected by means of the Töpler pump as before, and analysed.

The results throughout were negative, and thus proved *a fortiori* that at the anæsthetising values chloroform does not depress the solubility of the respiratory gases in the blood.

As an example, an experiment with serum shaken up with a mixture of air and carbon-dioxide may be quoted :—

A volume of 500 c.c. of serum was exhausted as above described.

(*a*) A volume of 150 c.c. of this serum was poured into a 500 c.c. stoppered bottle, and shaken up with a mixture of air and carbon-dioxide.

Exhaustion and analysis of the gases in 70 c.c. gave the following results at 14° C. and 759 mm. :—

CO_2 41·2 c.c., O = 1·4 c.c. N = 5·4 c.c.

(*b*) A second volume of 150 c.c. of the exhausted serum treated exactly similar, but with 1·5 grammes of chloroform added, gave the following results from 70 c.c., at the same temperature and pressure :—

CO_2 41·4 c.c., O = 1·8 c.c. N = 6·4 c.c.

There was obviously a slight leakage of air, but the figures are sufficient to show that there is no appreciable change in the solubilities due to the presence of the chloroform.

Summary and Conclusions.

1. We believe that the experiments recorded above justify the conclusion that chloroform forms an unstable chemical compound or physical aggregation with the proteids experimented with, and that it is carried in the blood in such a state of combination. Since proteids build up the protoplasm of living cells, it appears to us probable that chloroform, and other anæsthetics, must form similar combinations with protoplasm, and that anæsthesia is due to the formation of such compounds which limit the chemical activities of the protoplasm. The compounds are unstable, and remain formed only so long as the pressure of the anæsthetic in the solution is maintained. Such compounds are formed not only by hæmoglobin but by serum proteid, and hence the position taken by the anæsthetic in hæmoglobin is not that of the respiratory oxygen. This is further shown by the fact that the oxygen-carrying power of hæmoglobin is not interfered with in presence of chloroform.

The effect of chloroform upon various forms of protoplasm will form the subject of future experiments.

The facts upon which we rely as proofs of the formation of a compound or aggregation between chloroform and serum proteid or hæmoglobin may be summarised as follows :—

(*a*) Chloroform has a much higher solubility in serum or hæmoglobin solutions than in saline or water.

(*b*) *Even in dilute solutions* at the same pressure the amount of chloroform dissolved in serum or hæmoglobin solution is considerably higher than in saline or water.

(*c*) The curve of pressures and concentrations in the case of water and saline is a straight line, while in the case of serum and hæmoglobin solution it is a curve, showing association at the higher pressures.

(*d*) In the case of serum, chloroform causes a marked opalescence, and also a slow precipitation at room temperature (15° C.), and at body temperature (40° C.) a rapid, though incomplete precipitation. In the case of hæmoglobin, 1·5—2 per cent. of chloroform causes a change of colour and commencing precipitation at room temperature, which becomes almost complete in the thermostat at 40° C., while 5 per cent. and over causes complete precipitation even at 0° C.

2. The relations between chloroform pressure and concentration in solution have been worked out throughout a long range, from below the anæsthetising values (8—10 mm.) to nearly saturation in the case of water, saline, and serum.

Attention may be drawn here to the important practical fact that with the same percentage of chloroform in the air breathed, serum or hæmoglobin, and therefore the blood, will take up much more chloroform than would water or saline under equal conditions. Thus at the anæsthetising pressure, and at 40° C., the coefficient of distribution in the case of water and saline is approximately 4·6, while that of serum is 7·3; at room temperature (15° C.) these coefficients become 8·8 and 17·3 respectively.

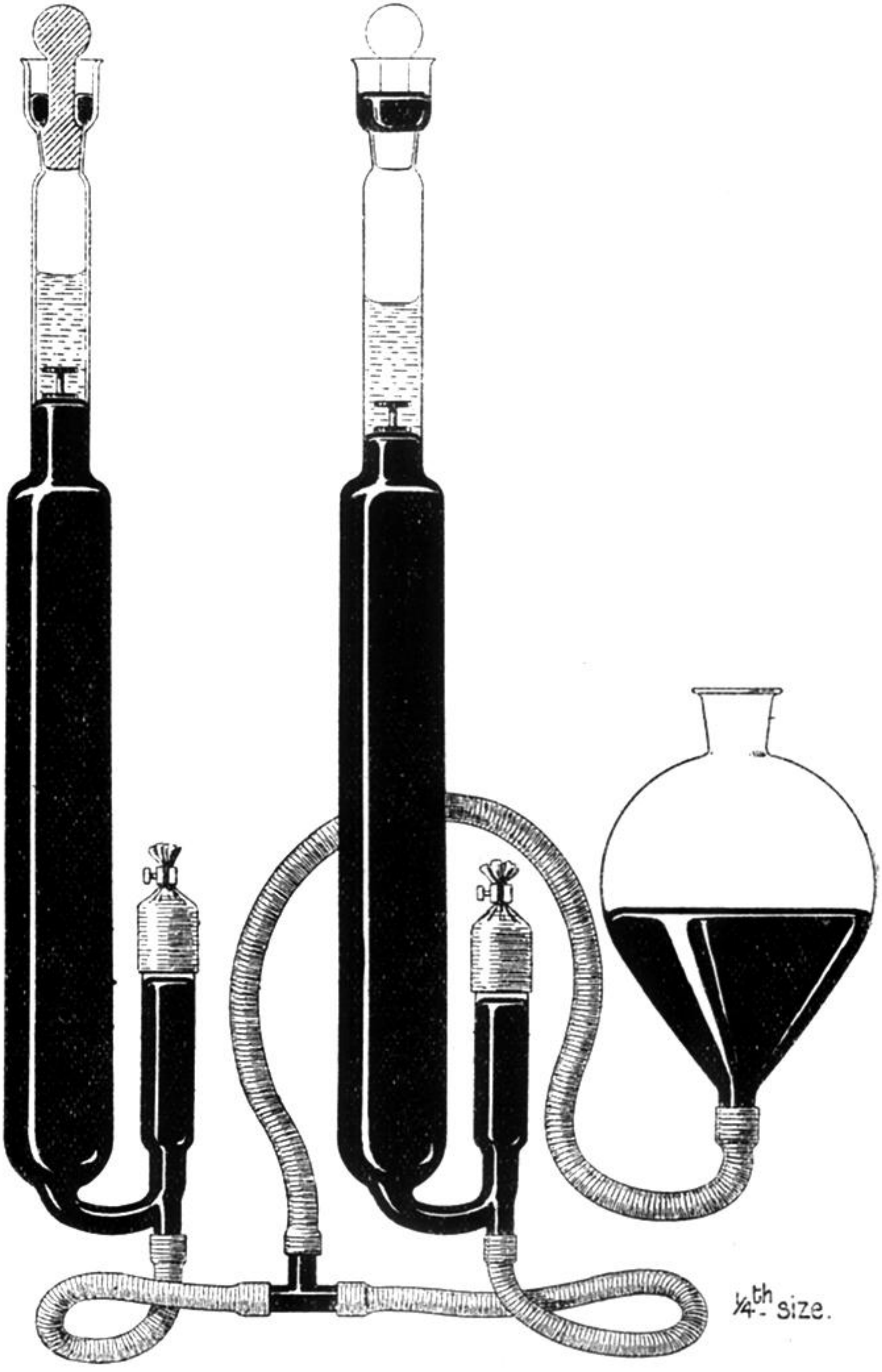


FIG. 1.—Diagram of differential Densimeter.

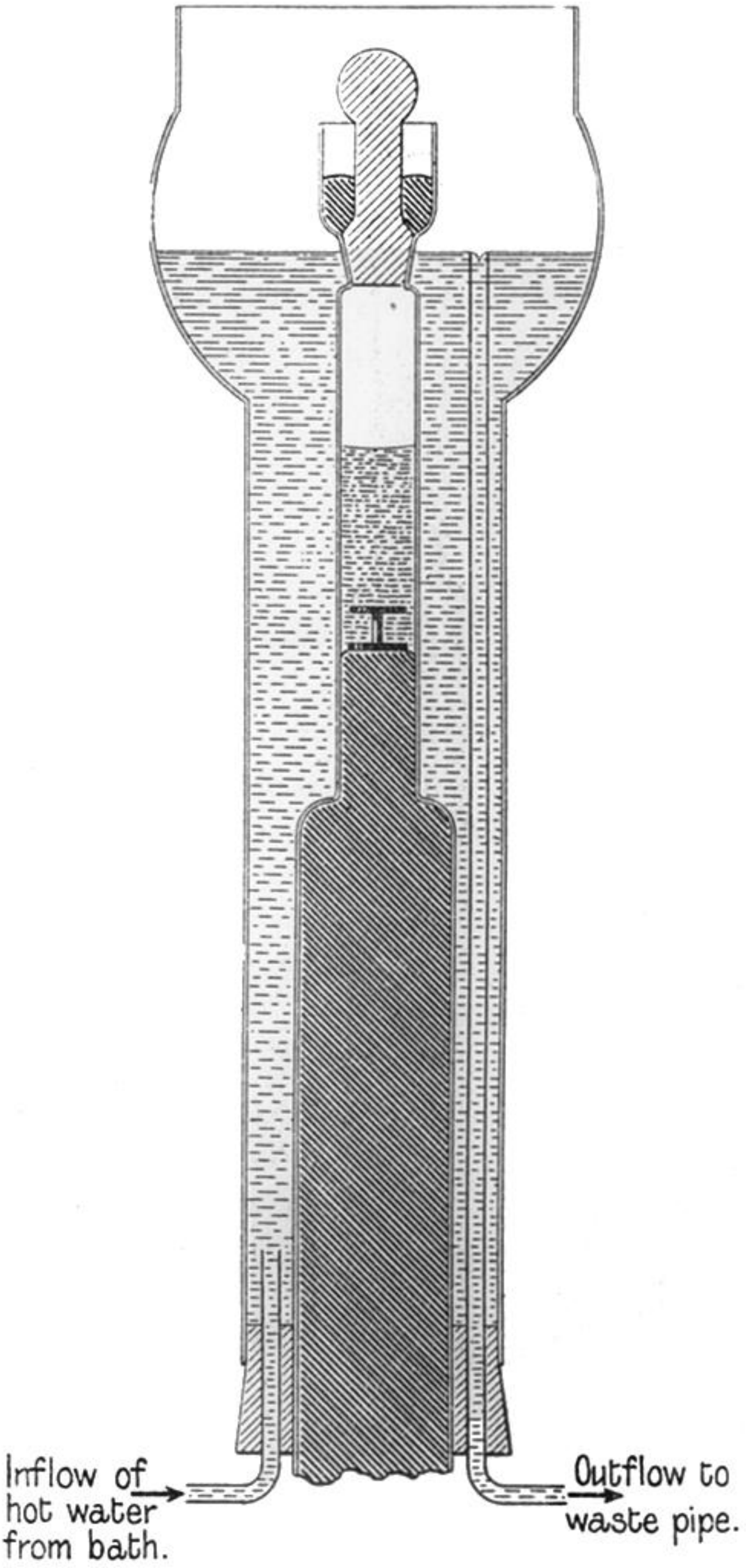


FIG. 2.—Section taken through the upper portion of one tube of the differential Densimeter and the hot-water jacket, showing the inflow and outflow tubes. Scale $\frac{1}{3}$.